Influence of Antibody Avidity on Glomerular Immune Complex Localization

SAMY S. ISKANDAR, MB, BCh, PhD, and J. CHARLES JENNETTE, MD

From the Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina

Immune-complex-mediated glomerulonephritis (IC-GN) was induced with daily intraperitoneal injections of horse apoferritin (HAF) for varying intervals in Swiss albino and BALB/c mice. Anti-HAF antibody avidity in plasma pools from mice with predominantly mesangial immune deposits was compared with avidity in plasma pools from mice with predominantly capillary wall deposits. Plasma from Swiss mice having predominantly mesangial deposits after 7 or 14 days of HAF had higher avidities than plasma from Swiss mice with predominantly capillary wall deposits after 14 days or more than 28 days of HAF. Plasma from BALB/c mice with exclusively mesangial deposits after 7 days of HAF had higher avidity than plasma from BALB/c mice with predominantly capillary wall deposits after 14 or more days of HAF. Therefore, there was a correlation between glomerular site of immune deposition and avidity of circulating anti-HAF antibodies, with higher avidity antibodies associated with mesangial immune deposits and lower avidity antibodies with capillary wall deposits. (Am J Pathol 1983, 112:155-159)

EVER SINCE Germuth[1] and Dixon[2] substantiated the role of immune complex (IC) deposition in the pathogenesis of serum sickness type glomerulonephritis (GN), investigators have been striving to elucidate quantitative and qualitative aspects of immune responses that determine the occurrence and nature of IC-mediated glomerular lesions. The avidity of antibodies contributing to circulating ICs has recently received much attention.[3-8] The reports cited deal with two different issues. The first is the influence of antibody avidity on an individual's susceptibility to GN.[3] The second issue is the role played by antibody avidity in determining the site within the glomerulus of nephritogenic IC deposition.[4-8] In this communication we present observations derived from experiments on two murine models of heterologous protein-induced IC-GN. These investigations indicate that animals with mesangial deposition of immune reactants have circulating antibodies of higher avidity (relative affinity) than their counterparts with predominantly capillary wall deposits.

Materials and Methods

Two-month-old male Swiss albino and BALB/c mice (GIBCO Animal Resources Labs, Grand Island, NY) were given daily intraperitoneal injections of 4 mg horse apoferritin (HAF) (Calbiochem-Behring Corporation, La Jolla, Calif) in saline for varying periods of time. At the time of sacrifice (1 day after the last HAF injection), heparinized blood from the retroorbital venous plexus and kidney tissue were collected. Plasma was stored at −70 C until used for assay, and kidney tissue was processed for light, immunofluorescence, and electron-microscopic studies as described previously.[9] For immunofluorescence microscopic examination, antiserums against mouse IgG, IgM, IgA, C3, and albumin and against horse ferritin were used. Pools of plasma were made from groups of mice selected on the basis of glomerular immune deposit localization as determined by immunofluorescence microscopy.

The affinity of antibodies determines the rate of dissociation of antigen-antibody complexes. When antibodies of high affinity are equilibrated with an excess of antigen, the amount of antigen excess does not substantially affect the chances of any particular antibody molecule being precipitated in complex

Accepted for publication March 8, 1983.

Address reprint requests to J. Charles Jennette, MD, Director, Immunopathology Laboratory, Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, NC 27514.

0002-9440/83/0805-0155$01.05

form by an agent such as polyethylene glycol, because once the antigen–antibody association takes place, dissociation is very slow to occur. On the other hand, in the case of low-affinity antibodies, because the rate of complex dissociation is high, increasing the concentration of antigen in the reaction medium enhances the chances of any antibody molecule colliding with an antigen molecule at any particular moment, thus increasing the chances of that molecule being precipitated in complex form. Therefore, the extent of change in the amount of antigen precipitated as a function of change in antigen concentration is greater for lower avidity antibodies than for higher avidity antibodies. Hence, comparison of the behaviors of different immune plasmas when incubated with varying concentrations of the appropriate antigen allows evaluation of their respective avidities. The Scatchard equation is one of a number of equations derived from the law of mass action[10] that can be used for that purpose.[11] Affinity, strictly speaking, refers to the strength of the bonds between a single antibody binding site and the corresponding antigenic determinant. Avidity, on the other hand, applies to the strength of interaction between multivalent antibodies and antigens. In the literature, however, the term "relative affinity" has frequently been used to describe measurements carried out on whole serum. To assay avidity (relative affinity) of anti-HAF antibodies in our system, serial dilutions of ^{125}I-HAF in 1:100 normal mouse plasma and phosphate-buffered saline (PBS) were prepared. Different HAF concentrations were used with Swiss and BALB/c plasmas so that the most discriminating Scatchard plots would be generated. Triplicate 40-μl aliquots of each serial dilution were placed in tubes to which 40 μl of a fixed dilution of the plasma pool to be assayed were added. This fixed dilution of plasma was selected so that pools whose avidities were to be compared bound approximately the same amount of ^{125}I-HAF in a quantitative anti-HAF antibody radioimmunoassay. The input radioactivity was counted, and the tubes were incubated for 16 hours on a rocking platform. Then 80 μl of 8% polyethylene glycol (PEG) in PBS was added to each tube. The tubes were shaken to ensure thorough mixing and stored at 4 C for 1 hour. After a single dilution with 2 ml of 4% PEG and centrifugation for 1 hour at 1500*g* and 4 C, the supernatants were aspirated, and the precipitated radioactivity was counted. Bound and free ^{125}I-HAF was calculated with the use

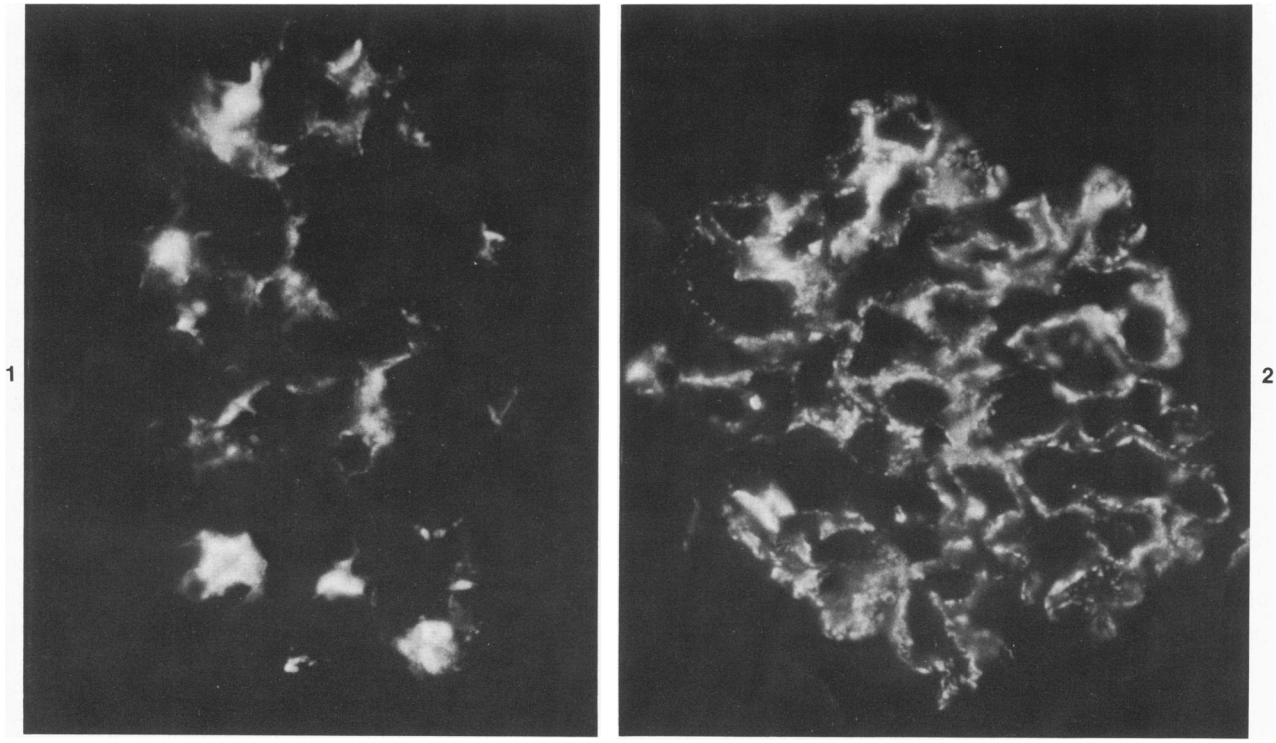


Figure 1 — Direct immunofluorescence photomicrograph of a glomerulus from a Swiss mouse that received HAF for 14 days and had a predominantly mesangial pattern of immune deposition. (Anti-mouse IgG, ×600) **Figure 2** — Direct immunofluorescence photomicrograph of a glomerulus from a Swiss mouse that received HAF for 14 days and had predominantly capillary wall immune deposits. (Anti-mouse IgG, ×600) (With a photographic reduction of 4%)

Table 1—Immunofluorescence Microscopic Findings

Plasma pool	Immunofluorescence microscopy				Avidity
	IgG	IgM	C3	HAF	
Swiss mice					
7-day* mesangial†	2.8/0.1‡	2.3/0	2.3/0	1.5/0	7.7×10^7 L/M
14-day mesangial	3.0/0.6	1.3/0	2.5/0.6	2.5/0.6	8.7×10^7 L/M
14-day capillary	0.3/3.7	1.3/0	0.5/3.3	1.7/2.3	5.6×10^7 L/M
≥28-day capillary	0/3.5	0.7/0	1.5/1.7	2/3.5	2.4×10^7 L/M
BALB/c mice					
7-day mesangial	1.8/0	2.2/0	1/0	0.5/0	2.4×10^8 L/M
≥14-day capillary	0.9/2.6	1.9/1.1	0.6/2.1	0.9/2.9	0.6×10^8 L/M

* Days of HAF administration.
† Predominant site of glomerular immune deposition.
‡ Averge mesangial/capillary intensity on a scale of 0–4.

of Farr's correction[12] and Scatchard plots constructed by plotting bound ^{125}I-HAF/free ^{125}I-HAF against bound ^{125}I-HAF. The absolute value of the slope of the line obtained gave the relative association constant (K). This assay system allowed comparison of avidities among different pools within each strain; but because HAF concentrations in assays of Swiss plasmas were different from those used to assay BALB/c plasmas, no comparison of relative affinities between the two strains was appropriate.

Avidity assays were performed on four Swiss plasma pools and two BALB/c plasma pools. The association constants determined for each of the four Swiss plasma pools were the mean of three assays. Swiss mouse plasma pools were from 4 mice receiving HAF for 7 days and having almost exclusively mesangial deposits, 4 mice receiving HAF for 14 days and having predominantly mesangial deposits, 3 mice receiving HAF for 14 days and having predominantly capillary loop deposits, and 2 mice receiving HAF for 28 or more days and having predominantly capillary loop deposits. BALB/c mouse plasma pools were from 5 mice receiving HAF for 7 days and having exclusively mesangial deposits and 7 mice receiving HAF for 14 or more days and having predominantly capillary loop deposits.

Results

Swiss Mice (Table 1)

Swiss mice with predominantly mesangial localization of immune deposits (Figure 1) had circulating anti-HAF of higher avidity than Swiss mice with predominantly capillary loop deposits (Figure 2). Swiss mice with predominantly mesangial deposits had almost the same anti-HAF avidity whether given HAF injections for 7 days ($7.7 \pm 0.7 \times 10^7$ L/M) or 14 days ($8.7 \pm 0.6 \times 10^7$ L/M). But Swiss mice given injections for 14 days and displaying predominantly capillary loop immune deposits had anti-HAF avidity of only $5.6 \pm 0.3 \times 10^7$ L/M. An even lower anti-HAF avidity of $2.4 \pm 0.5 \times 10^7$ L/M was found in Swiss mice with predominantly capillary wall deposits after 28 or more days of HAF. By light microscopy, 12 of the 13 Swiss mice used to form plasma pools had a proliferative GN (Figure 3). One Swiss mouse with mesangial immune deposits had no lesion by light microscopy. No consistent correlation could be discerned between the light-microscopic glomerular morphologic features and the site of immune deposits or anti-HAF avidity, but by electron microscopy the distribution of immune deposits noted by immunofluorescence microscopy corresponded to

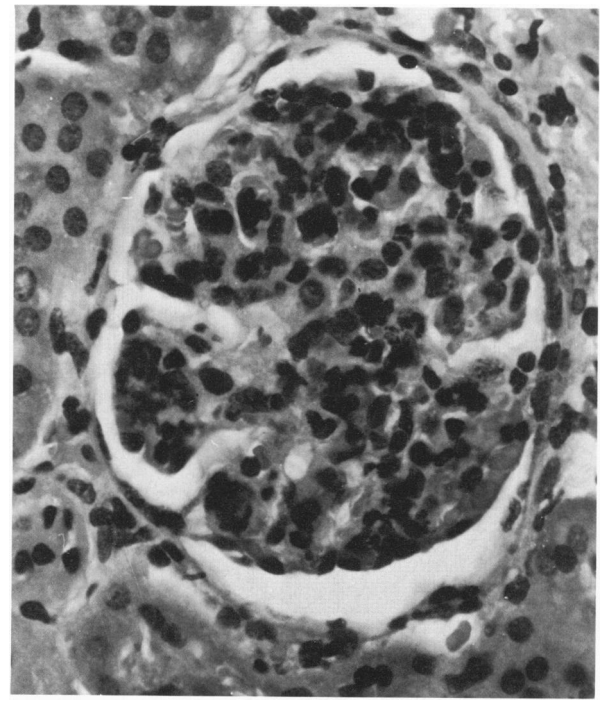
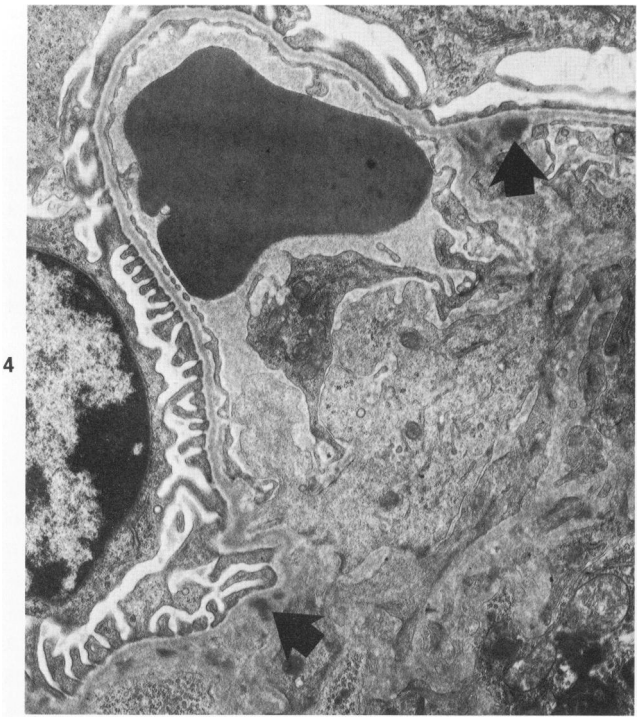


Figure 3—Photomicrograph of a glomerulus from a Swiss mouse that received HAF for 14 days and had a proliferative glomerulonephritis. (H&E, ×600) (With a photographic reduction of 12%)



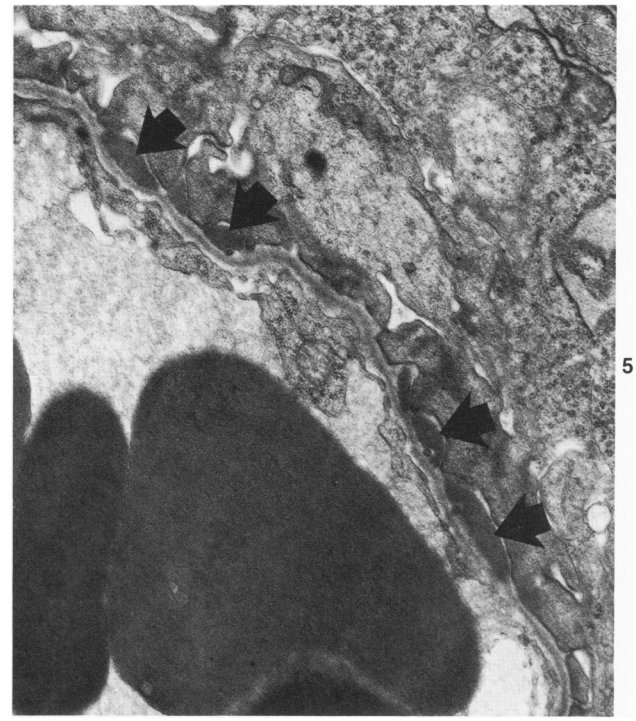


Figure 4—Electronmicrograph showing mesangial electron-dense deposits (*arrows*) in the glomerulus of a Swiss mouse that received HAF for 14 days and had predominantly mesangial immune deposits as seen by immunofluorescence microscopy. (× 10,000) **Figure 5**—Electronmicrograph showing subepithelial electron-dense deposits (*arrows*) in the glomerulus of a Swiss mouse that received HAF for 14 days and had predominantly capillary wall immune deposits as seen by immunofluorescence microscopy. (× 15,000) (With a photographic reduction of 9%)

electron-dense mesangial or subepithelial deposits (Figure 4 and 5).

BALB/c Mice (Table 1)

BALB/c mice given HAF for 7 days and having exclusively mesangial immune deposits had an anti-HAF avidity of 2.4×10^8 L/M, whereas BALB/c mice injected with HAF for 14 or more days and having predominantly capillary loop immune deposits had a much lower anti-HAF avidity of 0.6×10^8 L/M. By electron microscopy the former group had only mesangial electron-dense deposits, while the latter had mesangial, subendothelial, and subepithelial electron-dense deposits. Even though substantial mesangial immune deposition had occurred in the BALB/c mice given HAF for 7 days, no definite light-microscopic lesions were identified. All 7 BALB/c mice that received HAF for 14 or more days had marked proliferative glomerulonephritis, including one with numerous globally necrotic glomeruli.

Discussion

The role of antibody avidity as a determinant of site of glomerular IC localization has been repeatedly investigated in recent years. The earliest study addressing this point was that published by Kuriyama in 1973.[4] In this study, in 4 rabbits chronically immunized with egg albumin a lesion developed that was somewhat similar to human membranous glomerulopathy except for the presence of mesangial and subendothelial dense deposits in addition to subepithelial deposits classically seen in this entity. These animals were shown, using Farr's method, to produce antibodies of relatively lower avidity, compared with 2 other animals in which mesangioproliferative GN developed with electron-dense deposits essentially confined to the mesangium.

In 1978 Koyama et al[5] formed ICs *in vitro* with antibodies of different affinities, as determined by equilibrium dialysis. These complexes were injected intravenously into C57BL mice. Mice given "high affinity" complexes showed glomerular hypercellularity associated with mesangial deposits visualized both by immunofluorescence and electron microscopy. On the other hand, mice given "moderate affinity" complexes displayed the presence of immune reactants both in the mesangium and in the subendothelial area but not in the subepithelial compartment.

More recently, Germuth et al[6,7] used a mouse model of passive IC-GN and showed that egg albumin-anti-egg albumin complexes induced a mesangiopathy if they were formed with highly avid antibodies, whereas a proliferative GN with subepithelial deposits resulted when the complexes were

formed with poorly avid antibodies. In his studies Germuth defined the avidity of antibodies by the extent of solubility of ICs formed at equivalence in a pH 4 citrate buffer. Devey and Steward,[8] working with mouse lines producing high and low avidity antibodies, respectively, found that during IC-GN the high avidity line displayed predominantly mesangial immune deposits and the low avidity line had predominantly capillary wall deposits.

Our results are thus in accord with previous reports. The comparison of antibody avidities over time in the two models suggests that continued antigenic stimulation reduces the avidity of the antibodies produced. This could be explained by the progressive recruitment of populations of antibody-producing cells which synthesize antibodies with less and less adequate fits for the determinants on the HAF molecule.

One interpretation of the relationship between antibody avidity and site of glomerular IC localization is that relatively avid antibodies form large ICs, which localize in the mesangium. Antibodies of low avidity, on the other hand, form smaller complexes, which successfully achieve a capillary wall localization. This straightforward explanation should, however, be taken with reservation, because there is no guarantee that the ICs remaining in the circulation are representative of ICs deposited in the glomeruli. Indeed, evidence has been brought forth in two publications[13,14] that this might not be the case. Steward[13] found that after GN induction the renal eluate from both high and low avidity antibody producing lines contained what he considered to be high avidity antibodies. Similarly, Winfield et al[14] reported that anti-native-DNA antibody avidity was significantly lower in lupus patients with active nephritis as compared with those without nephritis. However, anti-native-DNA eluted from glomeruli of nephritic patients was highly avid in all cases studied. These reports raise the possibility that only selected populations of circulating ICs lodge in the glomeruli.

Another possible explanation of the observations under consideration would be that under conditions of high antibody avidity ICs once formed rarely dissociated and deposited from the circulation into the mesangium, while under conditions of low avidity ICs had a high rate of dissociation predisposing to independent penetration of antibody and antigen into capillary walls and resultant *in situ* formation.

In summary, the data presented substantiate a correlation between the glomerular site of IC deposition and the average avidity of circulating antibodies available to participate in the formation of these ICs. Higher avidity antibodies were associated with mesangial immune deposits and lower avidity antibodies with capillary wall immune deposits.

References

1. Germuth FG Jr: A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. J Exp Med 1953, 97:257–282
2. Dixon FJ, Vazquez JJ, Weigle WO, Cochrane CG: Pathogenesis of serum sickness. Arch Pathol 1958, 65:18–28
3. Soothill JF, Steward MW: The immunopathological significance of the heterogeneity of antibody affinity. Clin Exp Immunol 1971, 9:193–199
4. Kuriyama T: Chronic glomerulonephritis induced by prolonged immunization in the rabbit. Lab Invest 1973, 28:224–235
5. Koyama A, Niwa Y, Shigematsu H, Taniguchi M, Tada T: Studies on passive serum sickness: II. Factors determining the localization of antigen-antibody complexes in the murine renal glomerulus. Lab Invest 1978, 38:253–262
6. Germuth FG Jr, Rodriguez E, Lorelle CA, Trump EI, Milano L, Wise O'L: Passive immune complex glomerulonephritis in mice: Models for various lesions found in human disease: I. High avidity complexes and mesangiopathic glomerulonephritis. Lab Invest 1979, 41:360–365
7. Germuth FG Jr, Rodriguez E, Lorelle CA, Trump EI, Milano L, Wise O'L: Passive immune complex glomerulonephritis in mice: Models for various lesions found in human disease: II. Low avidity complexes and diffuse proliferative glomerulonephritis with subepithelial deposits. Lab Invest 1979, 41:366–371
8. Devey ME, Steward MW: The induction of chronic antigen-antibody complex disease in selectively bred mice producing either high or low affinity antibody to protein antigens. Immunology 1980, 41:303–311
9. Iskandar SS, Jennette JC, Wilkman AS, Becker RL: Interstrain variations in nephritogenicity of heterologous protein in mice. Lab Invest 1982, 46:344–351
10. Steward MW: Affinity of the antibody-antigen reaction and its biological significance, Immunochemistry: An Advanced Textbook, Edited by LE Glynn, MW Steward. Chichester, John Wiley and Sons, 1977, pp 233–262
11. Chia D, Nasu H, Yamagata J, Barnett EV: Avidity indices of anti-IgG antibodies in disease. Arth Rheum 1981, 24:803–808
12. Minden P, Farr RS: Ammonium sulphate method to measure antigen-binding capacity, Handbook of Experimental Immunology, Edited by DM Weir. Oxford, Blackwell Scientific publications, 1978, pp 13.1–13.22
13. Steward MW: Chronic immune complex disease in mice: The role of antibody affinity. Clin Exp Immunol 1979, 38:414–423
14. Winfield J, Faiferman I, Koffler D: Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus: Association of high avidity antinative DNA antibody with glomerulonephritis. J Clin Invest 1977, 59:90–96